AMENDMENTS TO THE SPECIFICATION

Without prejudice, please amend the Specification as described below.

Please amend Paragraphs **[0050]** through **[0055]** as follows:

~~**[0050]** FIGs. 21A-E show recovery of mRNA versus individual subjects.~~

[0051~~0~~] FIG. 2~~2~~1A is a graph showing percent recovery of standard RNA among various subjects.

[0052~~1~~] FIG. 2~~2~~1B is a graph showing CD4 mRNA per µl of blood recovered among various subjects.

[0053~~2~~] FIG. 2~~2~~1C is a graph showing p21 mRNA per µl of blood recovered among various subjects.

[0054~~3~~] FIG. 2~~2~~1D is a graph showing FasL mRNA per µl of blood recovered among various subjects.

[0055~~4~~] FIG 2~~2~~1E is a graph showing LTC4S mRNA per µl of blood recovered among various subjects.

[0055] In FIGs. 22A-H, △ shows p21 mRNA for the control stimulation, ▲ shows p21 mRNA for the PMA+CaI stimulation, ◇ shows FasL mRNA for the control stimulation, and ◆ shows FasL mRNA for the PMA+CaI stimulation.

Please add new Paragraphs **[0055A]** through **[0055H]** following new Paragraph **[0055]**:

[0055A] FIG. 22A is a graph showing *in vitro* induction of mRNA in whole blood.

[0055B] FIG. 22B is a graph showing blood storage before *in vitro* stimulation.

[0055C] FIG. 22C is a graph showing *in vitro* induction of FasL mRNA among various subjects, by showing mRNA molecules/mL blood for both stimulation and vehicle control, respectively.

[0055D] FIG. 22D is a graph showing *in vitro* induction of p21 mRNA among various subjects, by showing mRNA molecules/mL blood for both stimulation and vehicle control, respectively.

[0055E] FIG. 22E is the same graph as FIG. 22C rotated until the regression line becomes horizontal.

[0055F] FIG. 22F is the same graph as FIG. 22D rotated until the regression line becomes horizontal.

[0055G] FIG. 22G graphs the same data as FIG. 22C, showing fold increase for individual subjects.

[0055H] FIG. 22H graphs the same data as FIG. 22D, showing fold increase for individual subjects.

Please replace Paragraphs **[0101]** through **[0107]** and **[0113]** through **[0122]** with the following Paragraphs, respectively:

[0101] SEQ ID NO ~~31~~ 1: 5'-T_{40}-GGGTG CTGTG CTTCT GTGAA C-3',

[0102] SEQ ID NO ~~34~~ 2: 5'-GCCCC CTCAC TCCCA AATTC CAAGG CCCAG CCCTC ACACA TTGTT CACAG AAGCA CAGCA CCC-3',

[0103] SEQ ID NO ~~35~~ 3: 5'-GTAAT ACGAC TCACT ATAGG GGGAC AGCCC CCTCA CTCCC AAA-3',

[0104] SEQ ID NO ~~36~~ 4: 5'-GAAGC GTGTG TCACT GTGTG TTTCC AAGGC CCAGC CCTCA CACAT TGTTC ACAGA AGCAC AGCAC CC-3',

[0105] SEQ ID NO ~~37~~ 5: 5'-GTAAT ACGAC TCACT ATAGG GGGAC GGAAG CGTGT GTCAC TGTGT GT-3',

[0106] SEQ ID NO ~~74-T7~~ 6: 5'-GTAAT ACGAC TCACT ATAGG GGGAC GCATT CCGCT GACCA TCAAT A-3',

[0107] SEQ ID NO ~~76-T40~~ 7: T_{40}-TCCAA CGAGC GGCTT CAC-3'.

[0113] SEQ ID NO ~~23~~ 8: 5'-AAATG CCACA CGGCT CTCA -3'

[0114] SEQ ID NO ~~24~~ 9: 5'-CAAGT GTCTT CGTGT CGTGG G-3'

[0115] SEQ ID NO ~~25~~ 10: 5'-AGCCC CCTCA CTCCC AAA-3'

[0116] SEQ ID NO ~~26~~ 11: 5'-AGCCC CCTCA CTCCC AAA-3'

[0117] SEQ ID NO ~~27~~ 12: 5'-FAM-CAGTG GCTAG TGGTG GGTAC TCAAT GTGTA CTT-TAMRA-3'

[0118] SEQ ID NO ~~28~~ 13: 5'-FAM-CCAAG GCCCA GCCCT CACAC A-TAMRA-3'

[0119] SEQ ID NO ~~29~~ 14: 5'- CAGG GACAA ATGCC ACACG GCTCT CACCA GTGGC TAGTG GTGGG TACTC AATGT GTACT TTTGG GTTCA CAGAA GCACA GCACC CAGGG-3',

[0120] SEQ ID NO ~~75~~ 15: 5'-CCACT GGATT TAAGC AGAGT TCAA-3'

[0121] SEQ ID NO ~~76~~ 16: 5'-TCCAA CGAGC GGCTT CAC-3'

[0122] SEQ ID NO ~~77~~ 17: 5'-FAM-CAGCG GCCAG TAGCA TCTGA CTTTG A-TAMRA-3'

Please replace Paragraphs **[0078]**, **[0100]**, **[0112]**, **[0136]**, **[0139]**, and **[0151]** with the following Paragraphs, respectively:

[0078] Preferred embodiments of the lysis buffer include 10 to ~~1e^{10}~~ $1x10^{10}$, more preferably ~~1e^{5}~~ $1x10^{5}$ to ~~1e^{10}~~ $1x10^{10}$, copies of spiked RNA per well. In preferred embodiments, the amount of control RNA used is at least enough to be detected, but not so much as to significantly interfere with the amount of target mRNA that is quantified. In preferred embodiments, the control RNA added to the lysis buffer is poly(A)$^{+}$ RNA. In particularly preferred embodiments where the sample being tested is human blood, the control RNA is not homologous to RNA present in human blood. In some preferred embodiments, the sequence of the control RNA is less than 90% homologous to the target mRNA, or has greater than 10% difference in length with the target mRNA. In other preferred embodiments, the sequence of the control RNA is less than 85% homologous to the target mRNA, or has greater than 5% difference in length with the target mRNA. In further embodiments, the sequence of the control RNA is less than 75% homologous to the target mRNA, or has greater than 2% difference in length with the target mRNA. In

alternative embodiments, the sequence of the control RNA is less than 65% homologous to the target mRNA, or has greater than 1% difference in length with the target mRNA. In one embodiment, control RNA may preferably be made by amplifying template oligonucleotides by means of PCR. Thus, forward primers (SEQ ID NOs ~~25, 26, 75, and 23~~ 10, 11, 15, and 8), reverse primers (SEQ ID NOs ~~24, 76, and 24~~ 9 and 16), and TaqMan probes (SEQ ID NOs ~~28, 77, and 27~~ 13, 17, and 12) can be used to amplify various control RNA oligonucleotides. Alternative embodiments comprise using a plurality of different target mRNAs to be quantified. Further embodiments comprise using a plurality of control RNAs.

[0100] Preparation of control RNA. In order to synthesize control RNA, template oligonucleotides (SEQ ID NOs ~~34 and 36~~ 2 and 4) and cDNA from K562 cells (RNAture, Irvine, CA) were amplified with T7-forward primers (SEQ ID NOs ~~35, 37, and 74~~ 3, 5, and 6) and dT_{40} reverse primers (SEQ ID NOs ~~31, and 76~~ 1 and 7) with 30 cycles of 95°C denaturing for 30 sec, 55°C annealing for 10 sec, followed by 72°C extension for 20 sec, respectively. Oligonucleotides were purchased from IDT (Coralville, IA) or Proligo (Boulder, CO). The sequences were as follows ~~followed~~:

[0112] TaqMan real time PCR. Primers and TaqMan probes for control RNA were designed by Primer Express version 2.0 (ABI, Foster City, CA). For *bcr-abl,* we used published sequences. In some experiments, HYBsimulator (RNAture) was used to design reverse primers. The forward primers (SEQ ID NOs ~~25, 26, 75, and 23~~ 10, 11, 15, and 8), reverse primers (SEQ ID NOs ~~24, and 76~~ 9 and 16), and TaqMan probes (SEQ ID NOs ~~28, 77, and 27~~ 13, 17, and 12) were used to amplify control RNA. In order to determine the amounts of CD4 mRNA in blood samples, both CD4 and control RNA were analyzed in the different wells of PCR plates, rather than multiplex PCR in a single well. For β-actin, commercially available primers and probes were used (ABI). Into a 384 well PCR plate (ABI) were mixed: 2 μL of cDNA, 5 μL of TaqMan universal master mix (ABI), 1 μL of 5 μM of forward primers, 1 μL of 5 μM of reverse primers, and 1 μL of 2 μM TaqMan probe. PCR was conducted in an ABI PRISM 7900HT (ABI), using 1 cycle

of 95°C for 10 minutes, followed by 45 cycles of 95°C for 20 seconds, followed by 55°C for 20 seconds, and finally 60°C for 1 minute. The data were analyzed by SDS version 2.0 (ABI). In some experiments, the TaqMan assay was conducted directly in a GenePlate (Opticon, MJ Research). Oligonucleotides (SEQ ID NOs ~~34, 36, and 29~~ 2, 4, and 14) and PCR products were used as quantitation standards for control RNA. The sequences were as follows ~~followed~~:

[0136] Since the lysis buffer preferably contains a mixture of primers, two independent hybridization reactions took place simultaneously (FIG. 18A). One occurred between immobilized the immobilized oligo(dT) and the poly(A) tails of mRNA. The other hybridization reaction took place between specific primers and appropriate sites in mRNA (FIG. 18A (II)). Although the design of specific primers is critical, sufficient hybridization time made the assay more reproducible than that of primer hybridization during cDNA synthesis. It first appeared as though cDNA-mRNA duplex stayed in the solid surface via hybridization with immobilized oligo(dT) (Fig. ~~18A2~~ 18A (II)). Thus, cDNA was removed from the solid surface by heating at 95°C for 5 min. However, the amounts of amplified genes were unchanged to those of un-heated control (Fig. 18D inset). To test whether the cDNA-mRNA duplex was somehow removed from the solid surface, microplates were washed with water extensively after cDNA synthesis, and used for PCR directly. However, the target gene was successfully amplified from microplates with or without specific primers during the hybridization step (FIG. 18D). These data suggest that the specific primer-primed cDNA may be displaced with oligo(dT)-primed cDNA (FIGs. 18A (III, IV)). This makes the system advantageous; because the cDNA in solution is used for gene quantitation, the microplate itself can be used as a cDNA bank for validation, storage, and future use.

[0139] Maintaining the stability of whole blood after drawing blood is a prime concern. Thus, some commercial systems (PAX gene, PreAnalytix) uses special blood container, where cells are lysed immediately, and released RNA is stabilized for a relatively long period. However, manipulation of large volumes of lysate make entire

systems problematic. Moreover, because one of the goals of this project is to quantitate mRNA before and after gene induction processes *in vitro* (FIGs. ~~21A-H~~ 22A-H), heparinized whole blood was stored at 4°C and the changes in mRNA levels were examined. Although the levels of four native genes (CD4, p21, FasL, and LTC4S) were not stable after the blood draw, the levels became stable and constant after two hours whenever blood was stored at 4°C (FIG. 19B).

[0151] *In vitro* Responsiveness. In order to assess leukocyte responsiveness against phorbol 12-myristate 13-acetate (PMA) and calcium ionophore A23187 (CaI) (Sigma) as a model system, the levels of p21 and FasL mRNA was quantitated (FIG. 22). In other preferred embodiments, various types of mRNA can be analyzed in response to stimulation by various bioactive agents, including but not limited to, for example: radiation, ultraviolet, oxidative stress, ozone, temperature, mechanical stress, chemicals, peptides, hormones, proteins, antigens, antibodies, drugs, small molecule compounds, toxic materials, environmental stimuli, cell-cell communications, infectious agents, and allergens. Since the system used heparinized whole blood, rather than an isolated leukocyte suspension in artificial solution, the results reflected physiologically accurate conditions. In FIGs. 22A-H, △ shows p21 mRNA for the control stimulation, ▲ shows p21 mRNA for the PMA+CaI stimulation, ◇ shows FasL mRNA for the control stimulation, and ◆ shows FasL mRNA for the PMA+CaI stimulation. As shown in FIG. 22A, both p21 and FasL mRNA levels increased rapidly upon stimulation of PMA and CaI, and reached a plateau after 90-120 minutes with approximately a ten-fold increase. The increases in p21 were much faster than those of FasL (FIG. 22A). The levels of p21 were also increased slightly by incubation at 37°C without any stimulation, whereas FasL remained unchanged (FIG. 22A). Interestingly, the responsiveness was preserved even when heparinized whole blood was stored at 4°C for 21 hours (FIG. 22B), which provides wide flexibility for functional molecular analysis.